In The Specification

At page 132, please delete the second full paragraph at lines 5-11 and the fourth full paragraph at lines 19-20.

At page 132, lines 13-17, please amend the paragraph to read:

~~Figure 18~~ Figure 17 shows the structure of pMON25668. pMON25668 is the *Agrobacterium* plant transformation plasmid used to test expression of the wild-type *E.coli* threonine deaminase (IlvA) in soybean callus. The *phbA* gene on plasmid pMON25668 was not required for the expression of threonine deaminase.

At page 132, lines 22-24, please amend the paragraph to read:

~~Figure 20~~ Figure 18 shows the structure of pMON25628. pMON25628 was used for the overexpression in *E. coli* of the *A. eutrophus* PhbB reductase from the precA promoter.

At page 132, lines 26-29, please amend the paragraph to read:

~~Figure 21~~ Figure 19 shows the structure of pMON25629. pMON25629 was used for the overexpression in *E. coli* of the *A. eutrophus* PhbC synthase from the ptac promoter.

At page 133, lines 1-3, please amend the paragraph to read:

~~Figure 22~~ Figure 20 shows the structure of pMON25636. pMON25636 was used for the overexpression in *E. coli* of the *A. eutrophus* PhbA β-keto-thiolase from the ptrc promoter.

At page 133, lines 5-8, please amend the paragraph to read:

~~Figure 23~~ Figure 21 shows the structure of pMON25626. pMON25626 was the *Agrobacterium* plant transformation vector containing the genetic elements for seed-specific, plastid-targeted expression of PhbB reductase and PhbC synthase used to transform canola and soybean.

At page 133, lines 10-13, please amend the paragraph to read:

~~Figure 24~~ Figure 22 shows the structure of pMON25638. pMON25638 was the *Agrobacterium* plant transformation vector containing the genetic elements for seed specific, plastid-targeted expression of PhbA β-keto-thiolase used to transform canola and soybean.

At page 133, lines 15-18, please amend the paragraph to read:

~~Figure 25~~ Figure 23 shows the structure of pMON25822. This plasmid is derived from the broad host-range vector pBBR1MCS-3 (Kovach, 1994), and expresses *A. eutrophus bktB* from the *A. eutrophus bktB* promoter. The *bktB* promoter is included on pMON25728, discusses in example 8.

At page 166, lines 10 to page 167, line 4, please amend the paragraph to read:

In order to test the expression and enzymatic activity of the cloned E. *coli* biosynthetic threonine deaminases in plant cells, several plant expression plasmids were constructed for transient expression in tobacco leaf protoplast. The plant expression plasmids contain all the necessary elements for plant cell expression, including a promoter (i.e., e35S (Odell et al., 1985), the 5' untrans-lated leader sequence within the e35S promoter, a coding sequence, and a transcription termination and polyadenylation signal (i.e., E9 (Coruzzi et al., 1984)). The wild-type *E. coli* biosynethetic threonine deaminase (IlvA) encoded by the insert in pMON25663 (Figure 13) and the three mutant, deregulated *E. coli* biosynthetic threonine deaminases encoded by the inserts in pMON25686 (Figure 14), pMON25687 (Figure 15), and pMON25688 (Figure 16) containing the IlvA 219 (L447F), IlvA466 (L481F), and IlvA219/466 (L447F/L481F) mutations, respectively, were cloned downstream of the e35S constitutive plant promoter (Odell et al., 1985) and targeted to chloroplasts using a translational fusion to the *Arabidopsis* rubisco small subunit chloroplast transit peptide (ArabSSU1A; Stark et al., 1992). The four plasmids containing the various threonine deaminase-encoding DNAs were independently electroporated into tobacco leaf protoplasts by the method of Hinchee et al. (1994). Tobacco protoplasts electroporated with the various DNA's were sonicated to lyse the protoplasts. ~~Figure 17 shows the~~ The expression of the various IlvA enzymes was determined by Western blot analysis using rabbit polyclonal antibodies generated to the wild-type IlvA. Enzyme activity was monitored in

extracts of lysed protoplasts in the presence and absence of isoleucine as described above, and is reported in Table 4.

At page 167, line 15 to page 168, line 5 please amend the paragraph to read:

~~As shown in Figure 17, the~~ <u>The</u> "no DNA" control tobacco protoplasts did not exhibit any immunoreactive bands corresponding to threonine deaminase by Western blotting. In contrast, extracts of lysed tobacco protoplasts into which plasmids pMON25663, pMON25686, pMON25687, and pMON25688 were electroporated contained immunoreactive bands corresponding to the predicted molecular weight of the threonine deaminase encoded by the inserts of the respective plasmids. The enzymatic activity data reported in Table 4 demonstrate that both wild-type and deregulated threonine deaminases exhibit their predicted isoleucine deregulated enzymatic activities when expressed in plant cells based upon the results reported in the section entitled "Biochemical Analysis of Wild-type and Mutant *E. coli* Threonine Deaminases" of Example 2.

At pages 168, line 19 to page 169, line 3, please amend the paragraph to read:

Soybean callus tissue was transformed with binary Ti plasmid pMON25668 (~~Figure 18~~ <u>Figure 17</u>). Plasmid pMON25668 contains the e35S-expressed, plastid-targeted (ARABSSU1A) *E. coli* wild-type *ilv*A threonine deaminase gene, the e35S-expressed, plastid-targeted (ARABSSU1A) *A. eutrophus phb*A (β-ketothiolase) gene, and the e35s-expressed neomycin phosphotransferase type II (NptII) gene for kanamycin resistance. pMON26668 contains two border sequences for T-DNA transfer into the plant chromosome. The right border sequence is the only border required for T-DNA transfer into the plant; however, including the left border sequence terminates T-DNA integration at that point, thereby limiting the unnecessary incorporation of "extra" DNA sequences into the plant chromosome. pMON25668 also contains the minimum sequence of ori-322 for replication and ~~maintence~~ <u>maintenance</u> in *E. coli* as well as ori-V of the broad host origin RK2 for replication in *Agrobacterium*. TrfA is supplied in trans for proper replication in *Agrobacterium*. Further details of these vectors can be found in Glick et al., (1993) and the references cited therein.

At page 172, lines 1-4, please amend the paragraph to read:

Kanamycin-resistant calli were analyzed by Western blotting and enzymatic assays as described above to determine if *E. coli* IlvA was expressed and exhibited enzymatic activity in stably transformed soybean cells. ~~The results are shown in Figure 19.~~

At page 172, lines 6-12, please amend the paragraph to read:

~~The data in Figure 19 demonstrate that in~~ In four transformed calli (i.e., 518-4, 518-15, 518-16, and 518-17), the *E. coli* IlvA biosynthetic threonine deaminase was detectable by Western blot analysis. Enzymatic activity of the IlvA was also confirmed in the same transformed calli, correlating with the positive Western events. These results demonstrate that the *E. coli* IlvA can be expressed and maintain enzymatic activity in stably transformed plant tissue.

At page 180, line 25 to page, 181, line 15, please amend the paragraph to read:

As noted above, A. *eutrophus* can produce P(3HB)-co-3HV) copolymer when provided with an appropriate C5 precursor. Slater et al. (1988) demonstrated the presence of genes other than *phbA* encoding β-ketothiolase activity in *A.eutrophus*. One of these β-ketothiolases, designated BktB herein, was encoded on plasmid pBK6. A. *eutrophus* BktB was obtained from *E. coli* DH5α transformed with plasmid pMON25754. pMON25754 was produced from the *A. eutrophus* pBK6 clone (Slater et al., 1988) by deleting a 5.0kb XhoI fragment (there is an additional XhoI site approximately 5.0kb 5' to the XhoI site shown in Figure 1 of Slater et al.). The pBK6 clone was produced by Slater et al. (1988) from A. *eutrophus* H16, i.e., ATCC accession number 17699, which is publicly available from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852. BktB was overexpressed from its native promoter on the high copy plasmid pMON25754 by growing the cells overnight at 37°C to stationary phase in LB broth containing ampicillin (100µg/ml). A. *eutrophus* PhbA was obtained from *E. coli* DH5α transformed with pMON25636 (~~Figure 22~~ Figure 20) by growing the cells at 37°C in LB broth containing ampicillin (100µg/ml) to early log phase and then inducing with IPTG. Cells were harvested after two hours of further growth. PhbA and BktB protein extracts

were prepared by pelleting the cells, resuspending in 50mM KPi and 5% glycerol, sonicating, and removing cellular debris by centrifugation.

At page 195, line 19 to page 196, line 8, please amend the paragraph to read:

Maps of pMON25628, pMON25629, and pMON25636 are shown in Figures ~~20-22~~ 18-20, respectively. pMON25728, a derivative of pBK6 (Slater et al., 1988), was created by first subcloning a 5.5Kb NotI/EcoRI fragment of pBK6 into pBluescript KS+ (Stratagene), creating pMON25722. pMON25722 was subsequently digested with BglII and EcoRI, releasing a 1.1 Kb fragment. The remaining vector was blunt ended with Klenow and religated, creating pMON25724. Finally, pMON25724 was partially digested with NcoI, and digested to completion with SacI, releasing a 0.9Kb fragment. The remaining vector was blunt ended with Klenow and religated, creating pMON25728.

At page 205, lines 4-11, please amend the paragraph to read:

pMON25779 was constructed as follow. pJM9131 (Kidwell et al., 1995) was digested with Stu I and the vector fragment was re-closed, thereby deleting a fragment of approximately 1Kb that is required for production of the PhbA β-ketothiolase. The resulting plasmid, designated pMON25748, was digested with Bsa AI and Eco RI, and the fragment encoding a portion of *phbC* and the entire *phbB* gene was used to replace the Bsa AI-Eco RI fragment encoding a portion of *phbC* in pMON25629 (~~Figure 21~~ Figure 19), producing pMON25779. pMON25779 encodes phbCB under Ptac promoter control.

At page 205, lines 12-21, please amend the paragraph to read:

pMON25822 (~~Figure 25~~ Figure 23) contains a fragment of ~~apporoximately~~ approximately 1.8 Kb encoding *A. eutrophus bktB* cloned into in the vector pBBR1MCS-3 (Kovach et al., 1994). pMON25822 was constructed as follows. pMON25765 is a pBluescript KS+ derivative that contains ~~A~~ a 1.8 Kb Fragment encoding *bktB*. This plasmid is constructed by digesting pMON25728 (discussed above) partially with Sal I, then completely with Xho I, and cloning the resulting 1.8 Kb fragment into pBluescript KS+ that has been digested with Sal I and Xho I. The 1.8 Kb *bktB* fragment contains a total of 3 Sal I sites. pMON25765 was digested with Xho I and

XbaI, and the 1.8Kb fragment encoding bktB was ligated to pBBR1 MCS-3 digested with Xho I and XbaI.

At page 207, line 24 to page 208, line 16, please amend the paragraph to read:

Two *Agrobacterium* plant transformation vectors were constructed to introduce the entire PHB biosynthetic pathway from *A. eutrophus* into canola and soybean. The first plasmid, pMON25626 (~~Figure 23~~ Figure 21), comprises the genetic information for the expression of three genes in the transformed plant: *phbC, phbB* and CP4 EPSPS. Specifically, pMON25626 contains, ~~begining~~ beginning at the right border (RB) and continuing clockwise: the 7S β-conglycinin seed-specific promoter (Doyle et al., 1986: Slighton and Beachy, 1987) followed by the *Arabidopsis* small subunit of RUBP carboxylase chloroplast transit peptide (Arab-SSU1A; Stark et al., 1992) translationally fused via an Nco I site to the *phbC* gene, followed by the polyadenylation and transcription termination signal contained in E 9 3' (Coruzzi, et al., 1984; Morelli et al., 1985). The vector continues with the 7S promoter, Arab-SSI1A, *phbB*, and E9 3' termination signal, constructed as above. Additionally, pMON25626 contains the FMV promoter (Richine et al., 1987), followed by the petunia EPSPS (5-enol-pyruvylshikimate-3-phosphate synthase) chloroplast transit peptide (PEPSP; Shah et al., 1986) translationally fused to the CP4 EPSPS gene (PCT International Publication WO 92/04449; Padgette et al., 1996), and the *nos 3'* polyadenylation and transcription termination signal (Fraley et al., 1983). The CP4 EPSPS gene was used as the selectable herbicide resistance marker ("glyphosate selection") in the transformation procedure (Shah et al., 1986). The CP4 EPSPS enzyme is a glyphosate-resistant form of the EPSPS enzyme involved in aromatic amino biosynthesis in plants and bacteria which catalyzes the reversible reaction of shikimate-3-phosphate and phosphoenolpyruvate to produce 5-enolpyruvylshikimate-3-phosphate (Padgette et al., 1996).

At page 208, lines 17-24, please amend the paragraph to read:

The second plant transformation vector, pMON25638 (~~Figure 24~~ Figure 22), containing the *phbA* and the CP4 EPSPS genes, was constructed in a ~~mannersimilar~~ manner similar to that of pMON25626. The promoters, chloroplast transit peptides, and polyadenylation and transcription termination signals were identical to those described above for pMON25626. Both

pMON25626 and pMON25638 contain the identical border sequence and replication origins and functions as described for pMON25668 of Example 5 in the section entitled "Expression and Activity in Transformed Soybean Callus".

At page 217, lines 13-31, please amend the paragraph to read:

Glyphosate (0.05 mM-0.1 mM) was employed as a selectable marker (Hinchee et al., 1994) for both canola and soybean. Leaves of glyphosate-resistant canola and soybean transformants (designated R_0 generation) were screened for CP4 EPSPS expression by ELISA (Padgette et al., 1995). Seeds from R_0 CP4-positive plants were assayed enzymatically for either the PhbA β-ketothiolase or PhbA reductase, depending upon the plasmid used for transformation (data not shown). PHA synthase was not measured enzymatically; however, this gene is carried on the same genetic insert as the *phbB* reductase (see ~~Figure 23~~ Figure 21), and plants containing PhbB activity are likely to contain PhbC activity as well. Soybean and canola seed extracts were prepared by grinding the seeds to a fine powder in liquid N_2, washing with acetone three times, and filtering through Whatman 3MM paper. The seed extracts were dried at 37°C, one ml of extraction buffer (100mM KPi, pH7.4, 5mM $MgCl_2$, 1mM EGTA, 5mM DTT, 10% glycerol) was added, the mixture was vortexed, and the insoluble fraction was pelleted. The extracts were used for enzyme assays and to detect all PHB biosynthetic enzymes by Western blot analysis according to Nawrath et al. (1994). Immunoreactive bands corresponding to each of these enzymes were detected in extracts of seeds of both plants (data not shown).